Effects of supplemental β-carotene on mucosal IgA induction in the jejunum and ileum of mice after weaning.

AUTHOR(S):

Nishida, Keita; Sugimoto, Miki; Ikeda, Shuntaro; Kume, Shinichi

CITATION:

Nishida, Keita ...[et al]. Effects of supplemental β-carotene on mucosal IgA induction in the jejunum and ileum of mice after weaning.. The British journal of nutrition 2014, 111(2): 247-253

ISSUE DATE:

2014-01-28

URL:

http://hdl.handle.net/2433/189086

Effects of supplemental β-carotene on mucosal IgA induction in the jejunum and ileum of mice after weaning

Keita Nishida, Miki Sugimoto, Shuntaro Ikeda, and Shinichi Kume*

Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

Corresponding author: Shinichi Kume,
Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho,
Sakyo-ku, Kyoto 606-8502, Japan
Tel) +81-75-753-6325, Fax) +81-75-753-6345, E-mail: kume@kais.kyoto-u.ac.jp

Key words: Supplemental β-carotene: Jejunum: Ileum: IgA induction: Weanling mice

Abbreviations: ASC, antibody-secreting cells; pIgR, polymeric-Ig receptor; RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor.

* Corresponding author: Professor S Kume, fax +81 75 753 6345,
e-mail: kume@kais.kyoto-u.ac.jp

Abstract

An adequate immune system is required to prevent diarrhoea in neonates, and IgA provides protection against microbial antigens on mucosal surfaces. Although β-carotene supplementation has been expected to enhance the retinoic acid (RA)-mediated immune response in neonates, the exact mechanism of β-carotene for enhancing mucosal IgA production in small intestine is still unclear. We investigated the effect of supplemental β-carotene to weanling mice on IgA concentrations, the numbers of IgA antibody-secreting cells (ASC) and mRNA expression of IgA C-region, CCL25, retinoid X receptor (RXR)α, RA receptor (RAR)α and RARγ in jejunum and ileum of mice. Weanling mice were fed rodent feed or 50 mg/kg β-carotene-supplemented rodent feed for 7, 14 or 21 days. IgA concentrations and the numbers of IgA ASC in jejunum and ileum of mice increased markedly with age, and supplemental β-carotene increased IgA concentration, the numbers of IgA ASC and mRNA expression of IgA C-region, CCL25 and RARγ in jejunum after 14 and 21 days of treatment. Supplemental β-carotene increased the numbers of IgA ASC in ileum after 14 and 21 days of treatment, but IgA concentration in ileum was not affected by β-carotene supplementation. The mRNA expression of RXRα and RARα in jejunum and RXRα and RARγ in ileum after 21 days of treatment was enhanced by β-carotene supplementation. These results indicate that β-carotene supplementation to weanling mice is effective to enhance mucosal IgA induction in jejunum or ileum and that the effects are mainly due to the RA-mediated immune response.

Introduction

Mortality and morbidity of neonates continue to be major problems in humans and animals, and their most common disease is diarrhoea. Successful neonatal health depends on many factors related to management and nutrition, but improvement of the immune system is required to prevent diarrhoea. IgA is the most abundant Ig isotype in mucosal secretions and provides protection against microbial antigens on mucosal surfaces[1-3], and IgA antibodies produced from IgA antibody-secreting cells (ASC) in intestines and mammary glands are secreted mainly as dimers[1,4]. Supplemental vitamin A and β-carotene enhance the immune system of neonates[5-9], and supplementation of vitamin A decreases diarrhoea and mortality in malnourished children[10,11]. In previous studies[12,13], β-carotene supplementation to maternal mice during pregnancy and lactation increased the number of IgA ASC in mammary glands and ileum of lactating mice and enhanced IgA transfer from maternal milk to neonatal mice. However, very few IgA ASC were detected in jejunum and ileum of neonatal mice at 14 days of age and most IgA in neonatal mice may be derived from milk IgA[12]. Little intestinal secretions of IgA in mice at weaning could be found, and IgA rose rapidly after weaning and reached a maximum concentration at 10 weeks of age[14]. Thus, the enhancement of mucosal IgA induction is important for maintaining a normal immune system in neonates.

Gut-associated lymphoid tissue is the largest immunologic tissue in the body. Peyer's patches are the main site for the generation of IgA^+ B cells, and plasmablasts differentiated by IgA^+ B cells home preferentially in the gut lamina propria through the thoracic duct and blood by the expression of homing ligands and receptors[1-3]. Chemokines are transmembrane proteins that play important roles in innate and acquired immunity[15-17], and chemokine ligand CCL25 is selectively expressed in small intestine and its receptor CCR9 is expressed by almost all T cells in small intestine and a fraction of IgA ASC[18]. The interplay of CCL25 and CCR9 is likely to have a significant role in the recruitment of developing thymocytes[19], and the selective expression of

CCL25 in the small bowel underlies the homing of $CCR9^+$ intestinal memory T cells to the small bowel[20].

Vitamin A metabolite, all-*trans* retinoic acid (RA), plays important roles in gut immunity and RA is necessary for the imprinting of gut-homing specificity on T cells and the induction of gut-homing receptors on B cells and IgA ASC[16,17]. Several effects of carotenoids are thought to be mediated by their metabolism to vitamin A and subsequent mediation of RA receptor (RAR) and retinoid X receptor (RXR) response pathways[7]. RA is a highly potent activator of RAR and RXR and influences the transcription of various retinoid response genes by activation of these receptors[7,21,22]. RA regulates the apoptosis of T lymphocytes through interplay between RAR and RXR, but RARα and RARγ induce opposite effects during thymic selection[23]. The expression of RAR and RXR isotypes varies greatly in different tissues, but human lymphocytes express RXRα, RARα and RARγ[3,24]. Supplemental β-carotene increased mRNA expression of IgA C-region and the number of IgA ASC in ileum of lactating mice, and these effects may be mainly due to the RA-mediated immune response[12]. However, the exact mechanism of β-carotene for enhancing mucosal IgA induction in the intestines of neonates is still unclear, although β-carotene supplementation has been expected to enhance RA-mediated immune response in neonates[12, 13].

We investigated the effect of supplemental β-carotene to weanling mice on IgA concentration, the numbers of IgA ASC and mRNA expression of IgA C-region, CCL25, RXRα, RARα and RARγ in jejunum and ileum of mice. The present study demonstrated that β-carotene supplementation to weanling mice is effective to enhance mucosal IgA induction in jejunum or ileum, because supplemental β-carotene increased IgA concentration, the numbers of IgA ASC and mRNA expression of IgA C-region in jejunum and also the number of IgA ASC in ileum. These effects may be mainly due to the RA-mediated immune response owing to the increased mRNA expression of CCL25, RXRα, RARα and RARγ in jejunum and RXRα and RARγ in ileum.

Materials and Methods

Animals and diets

Male weanling ICR mice at 21 days of age (*n* 44) were purchased from Clea Japan (Tokyo, Japan). They were housed in individual polycarbonate cages and maintained in an air-conditioned room (24±2℃) under controlled lighting conditions (light-dark cycle, 14:10 h). They received humane care in accordance with the "Regulation on Animal Experimentation at Kyoto University" (Animal Research Committee, Kyoto University, revised 2007).

Weanling mice were randomly allocated to the control or β-carotene group at 21 days of age. Mice in the control group were fed rodent feed (Oriental Yeast, Tokyo, Japan) for 7 (*n* 8), 14 (*n* 8) or 21 (*n* 6) days, and mice in the β-carotene group were fed 50 mg/kg β-carotene-supplemented rodent feed for 7 (*n* 8), 14 (*n* 8) or 21 (*n* 6) days. The rodent feed contained a vitamin mix and vitamin A concentration was 1283 IU/100g, but β-carotene was not supplemented in the control group. In the β-carotene group, β-carotene was mixed with the rodent feed at 50 mg/kg. All mice were allowed free access to water and feed. Body weights and feed intake of mice were measured at 7.00 hours every day.

Sample collection

Blood samples from eight mice in the control and β-carotene groups after 7 and 14 days of treatment and six mice in the control and β-carotene groups after 21 days of treatment were obtained by cardiac puncture under anaesthesia with Avertin (2,2,2-tribromoethanol, Sigma-Aldrich Chemical, St Louis, MO, USA), and then jejunum, ileum and rectal faeces were removed after euthanasia by cervical dislocation. Samples of jejunum and ileum were immediately frozen in dry ice-cooled isopentane (2-methylbutane, Wako Pure Chemicals, Osaka, Japan) for immunohistochemical analysis or frozen in liquid nitrogen and stored at -80℃ for IgA

immunoassay and semi-quantitative RT-PCR. Blood samples were left to stand at room temperature for 1 h and then centrifuged at 3000 rpm for 15 min. The samples of serum and rectum faeces were stored at -20℃ until IgA analysis.

IgA immunoassay and immunohistochemical analysis

IgA immunoassay of serum, jejunum, ileum and faeces and immunohistochemical analysis of jejunum and ileum were determined as previously described[12]. IgA concentration was measured using the Mouse IgA ELISA Quantitation Kit (Bethyl Laboratories, Montgomery, TX, USA) and the ELISA Starter Accessory Package (Bethyl Laboratories) according to the manufacturer's instructions. The sections obtained by immunohistochemical analysis were examined under an epifluoresence microscope (BX50, Olympus, Tokyo, Japan), and the resulting images were analysed by Image J software (National Institute of Health, Bethesda, MD, USA). IgA-positive cells in jejunum and ileum were counted in lamina propria of villi in eight randomised villi from each mouse and shown as IgA ASC/unit area of the lamina propria of villi (unit=10000 μm^2).

Semi-quantitative RT-PCR

The mRNA expressions of IgA C-region, CCL25, RXRα, RARα and RARγ in the jejunum and ileum were examined by semi-quantitative RT-PCR as previously described[12]. The relative abundance of specific mRNA was normalised by the abundance of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The primer pairs and PCR conditions used for IgA C-region and GAPDH are the same as those in the previous study[12]. The primer pairs for CCL25 were as follows: forward: 5'-CCTTCAGGTATCTGGAGAGGAGATC-3', reverse: 5'-CAAGATTCTTATCGCCCTCTTCA-3'. The PCR cycles were as follows: after 95°C for 7 min to denature DNA, PCR was performed for thirty-three cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, then for one cycle at 72°C for 7 min. The primer pairs for RXRα were as

follows: forward: 5'-GAGCAGCACTGAGGATATCAAGC-3', reverse: 5'-GGTCAGGTCTTTGCGTACTGTCC-3. The PCR cycles were as follows: after 95°C for 7 min to denature DNA, PCR was performed for thirty-five cycles at 94°C for 1 min, 57°C for 1 min, 72°C for 1 min, then for one cycle at 72°C for 7 min. The primer pairs for RARα were as follows: forward: 5'-AGCACCAGCTTCCAGTCAGT-3', reverse: 5'-AGTGGTAGCCGGATGATTTG-3. The PCR cycles were as follows: after 95°C for 7 min to denature DNA, PCR was performed for thirty-seven cycles at 94°C for 1 min, 53°C for 1 min, 72°C for 1 min, then for one cycle at 72°C for 7 min. The primer pairs for RARγ were as follows: forward: 5'-GGGCAAGTACACCACGAACT-3', reverse: 5'-ATCCGCAGCATTAGGATGTC-3. The PCR cycles were as follows: after 95°C for 7 min to denature DNA, PCR was performed for thirty-seven cycles at 94°C for 1 min, 53°C for 1 min, 72°C for 1 min, then for one cycle at 72°C for 7 min.

Statistics

Data are expressed as mean values with their standard error. Data from body weight and feed intake were analysed by least squares ANOVA using the general linear model procedure of Statistical Analysis Systems (SAS Institute, Cary, NC, USA) [25]. The model was as follows:

$$Y_{ijk}=\mu+T_i+M_{(i)j}+D_k+TD_{ik}+e_{ijk}$$

where μ is the overall mean, T_i is the effect of treatment, $M_{(i)j}$ is the random variable of mice nested in treatment, D_k is the effect of sampling day, TD_{ik} is the interactions and e_{ijk} is the residuals. The general linear model procedure of Statistical Analysis Systems[25] was used to analyse the effects of treatment or time on some variables in mice. Significance was declared at $P<0.05$.

Results

IgA concentration in serum and tissues

Body weight gains and feed intake of mice during 21 days of treatment as well as those of mice during 7 and 14 days of treatment (data not shown) were similar between groups (Fig. 1). IgA concentrations in jejunum of the β-carotene group were significantly higher than those of the control group after 14 ($P<0.01$) and 21 ($P<0.05$) days of treatment, but IgA concentrations in serum, ileum and faeces of mice after 7, 14 and 21 days of treatment were not affected by treatment (Fig. 2). Compared with IgA concentrations of mice after 7 days of treatment, IgA concentrations in serum ($P<0.001$) and faeces ($P<0.01$) increased after 14 and 21 days of treatment. IgA concentrations in jejunum and ileum increased ($P<0.001$) with age.

IgA ASC in tissues

The numbers of IgA ASC in jejunum of the β-carotene group were significantly higher than those of the control group after 7 ($P<0.05$), 14 ($P<0.001$) and 21 ($P<0.001$) days of treatment (Fig. 3), and those in ileum of the β-carotene group were significantly higher than those of the control group after 14 ($P<0.001$) and 21 ($P<0.05$) days of treatment. The numbers of IgA ASC in jejunum and ileum of mice increased ($P<0.001$) with age.

Expression of mRNA in tissues

The mRNA expression of IgA C-region in jejunum of the β-carotene group was significantly higher ($P<0.01$) than that of the control group after 14 and 21 days of treatment, and the mRNA expression of CCL25 in jejunum of β-carotene group after 14 ($P<0.01$) and 21 ($P<0.05$) days of treatment was significantly higher than that of the control group (Table 1). The mRNA expression of RXRα in jejunum and ileum of the β-carotene group was significantly higher ($P<0.01$) than that of the control group after 21 days of treatment. The mRNA expression of RARα in jejunum

of the β-carotene group after 21 days of treatment was significantly higher (P<0.05) than that of the control group, but the mRNA expression of RARα in jejunum of the β-carotene group after 7 days of treatment was significantly lower (P<0.01). The mRNA expression of RARγ in jejunum of the β-carotene group after 14 and 21 days of treatment was significantly higher (P<0.05) than that of control group, and the mRNA expression of RARγ in ileum of β-carotene group after 21 days of treatment was significantly higher (P<0.05). There were no significant differences in mRNA expression of IgA C-region, CCL25 and RARα in ileum between groups.

Discussion

IgA antibodies in intestines are specific to antigens of the intestinal microflora and act to limit the penetration of commensal intestinal bacteria through the neonatal intestinal epithelium[26]. The intestinal mucosa of mice after weaning is exposed to a wide variety of exogenous antigens due to the sudden change of diet and is likely to increase Ig-secreting cells[14]. Mean faecal IgA concentration in mice at weaning was 3.1μg/g, but faecal IgA increased rapidly until 35 days of age and thereafter remained almost constant[27]. In the present study, however, IgA concentrations and the numbers of IgA ASC in jejunum and ileum of weanling mice increased rapidly with age, although IgA concentration in faeces and serum increased after 14 days of treatment and then remained constant.

Supplementation of vitamin A and carotenoids affects the immune-cell function during ontogenesis[28], and vitamin A-depleted mice show impaired IgA secretion in mucosal tissues of the small bowel [17]. In the present study, supplemental β-carotene at 50 mg/kg in the diet increased IgA concentration, the numbers of IgA ASC and mRNA expression of IgA C-region in jejunum of weanling mice after 14 and 21 days of treatment, but in ileum, supplemental β-carotene only increased the numbers of IgA ASC. On the other hand, supplementation of β-carotene at 50 mg/kg in the diet to maternal mice during pregnancy and lactation enhanced IgA

transfer from maternal milk to neonates owing to the increase in the numbers of IgA ASC in mammary glands and ileum of maternal mice, but β-carotene supplementation had no effect on the numbers of IgA ASC and mRNA expression of IgA C-region in jejunum[12]. In the previous[12,13] and present studies, however, β-carotene supplementation had no effect on IgA concentrations in serum and faeces of neonatal and weanling mice. Thus, β-carotene supplementation to weanling mice is effective to enhance mucosal IgA induction in small intestine owing to the increase in the numbers of IgA ASC in jejunum and ileum, but supplemental β-carotene at 50 mg/kg in the diet may have a predominant effect on jejunum IgA of weanling mice.

IgA in intestines are secreted mainly as dimers after incorporation of the J chain and association with polymeric-Ig receptor (pIgR), and the transport of IgA from IgA ASC into intestines was regulated by pIgR[1,4]. In the murine small intestine, mature isolated lymphoid follicles are inductive sites for the immune response and nodular lymphoid structures are observed in the distal small intestine[29]. On the other hand, supplementation of fructooligosaccharides to mice after weaning was more effective for enhancing IgA levels in jejunum rather than in ileum and colon, and supplemental fructooligosaccharides increased not only IgA but also pIgR expression in ileum and colon[30]. Additionally, coumestrol administration to maternal mice during pregnancy and lactation increased the number of IgA ASC in mammary glands, but had no effect on milk IgA and mRNA expression of pIgR in mammary glands[31]. These results indicate that the increased jejunum IgA of weanling mice may be due to the increase of mRNA expression of IgA C-region and pIgR expression by β-carotene supplementation.

Gut-associated lymphoid tissue dendritic cells rely on RA to induce IgA class switching, and RA is essential for the imprinting of gut-homing receptors on T and B cells and contributes to IgA production [16,17]. CCL25 plays essential roles in intestinal homing of IgA ASC primarily by mediating their extravasation into intestinal lamina propria[18]. RA is important to induce CCR9

on activated T cells, and blocking RA-receptors decreases the induction of gut-homing receptors[16]. In the present study, supplemental β-carotene increased mRNA expressions of CCL25 and RARγ in jejunum after 14 and 21 days of treatment and mRNA expressions of RXRα and RARα in jejunum and RXRα and RARγ in ileum after 21 days of treatment, although supplemental β-carotene decreased mRNA expression of RARα in jejunum after 7 days of treatment. These results imply that the enhanced mucosal IgA induction by β-carotene supplementation is mainly due to the RA-mediated immune response owing to the increased mRNA expression of RXRα, RARα and RARγ in jejunum and RXRα and RARγ in ileum, and β-carotene supplementation may influence the expression of chemokine receptor CCR9 on developing IgA ASC in jejunum.

In conclusion, the present study suggested that supplementation of β-carotene to mice after weaning is useful for enhancing mucosal IgA induction in intestines owing to the increase in IgA concentration, the numbers of IgA ASC and mRNA expression of IgA C-region in jejunum and the number of IgA ASC in ileum. Foods containing animal products and pro-vitamin A carotenoids are the primary source of vitamin A, but vitamin A deficiency is associated with an increased risk of death from common childhood infections[3,10]. Supplementation of β-carotene -enriched formulas may improve the immune system of neonates, because β-carotene was not detected in four out of eight brands of formula preparation and plasma β-carotene of formula-fed children was significantly lower than that of breast milk-fed children[6]. However, for children aged 1 to 3 years in the United States, the median vitamin A intake from foods and supplements was 1205μg of retinol activity equivalent/day and exceeded the tolerable upper level of 600μg/day but did not exceed the no-observed-adverse-effect level of 6000μg/day [32]. Further study is needed to clarify the optimal and toxic level of β-carotene in formulas to improve the immune system of neonates.

FINANCIAL SUPPORT

There was no financial support in the present study. Experimental β-carotene was kindly provided by Sceti Co. Ltd. (Tokyo, Japan).

CONFLICTS OF INTEREST

The authors have no conflicts of interest in this paper.

AUTHORSHIP

K. N., M.S., S.I., and S.K. designed the research; K.N conducted most of the research; K.N. and S.K contributed equally to the discussion and to the writing of the manuscript.

References

1. Fagarasan S & Honjo T (2003) Intestinal IgA synthesis: regulation of front-line body defences. *Nature Immunol* **3**, 63-72.
2. Sigmundsdottir H & Butcher EC (2008) Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking. *Nature Immunol* 9, 981-987.
3. Ertesvåg A, Naderi S & Blomhoff HK (2009) Regulation of B cell proliferation and differentiation by retinoic acid. *Semin Immunol* **21**, 36-41.
4. Wheeler TT, Hodgkinson AJ, Prosser CG, *et al.* (2007) Immune components of colostrum and milk- a historical perspective. *J Mammary Gland Biol Neoplasis* **12**, 237-247.
5. Bendich A & Shapiro SS (1986) Effect of β-carotene and canthaxanthin on the immune responses of the rat. *J Nutr* **116,** 2254-2262.
6. Sommerburg O, Meissner K, Nelle M, *et al.* (2000) Carotenoid supply in breast-fed and formula-fed neonates. *Eur J Pediatr* 159, 86-90.
7. Rühl R (2007) Effects of dietary retinoids and carotenoids on immune development. *Proc*

Nutr Soc **66**, 458-469.

8. Chew BP & Park JS (2004) Carotenoid action on the immune response. *J Nutr* **134,** 257S-261S.

9. Kume S & Toharmat T (2001) Effect of colostral β-carotene and vitamin A on vitamin and health status of newborn calves. *Livest Prod Sci* **68**, 61-65.

10. Mora JR & von Andrian UH (2009) Role of retinoic acid in the imprinting of gut-homing IgA-secreting cells. *Semin Immunol* **21**, 28-35

11. Stephensen CB, Moldoveanu Z & Gangopadhyay NN (1996) Vitamin A deficiency diminishes the salivary immunoglobulin A response and enhances the serum immunoglobulin G response to influenza A virus infection in BALB/c mice. *J Nutr* **126**, 94–102.

12. Nishiyama Y, Sugimoto M, Ikeda S, *et al.* **(**2011) Supplemental β-carotene increases IgA-secreting cells in mammary gland and IgA transfer from milk to neonatal mice. *Brit J Nutr* **105**, 24-30.

13. Nishiyama Y, Yasumatsuya K, Kasai K, *et al.* **(**2011) Effects of supplemental β-carotene with whey on IgA transfer from maternal milk and mucosal IgA induction in neonatal mice and calves. *Livest Sci* **137**, 95-100.

14. Van del Heijden PJ, Bianchi ATJ, Stok W, *et al.* (1988) Background (spontaneous) immunoglobulin production in the murine small intestine as a function of age. *Immunol* **65**, 243-248.

15. Morteau O, Gerard G, Lu O, *et al.* (2008) An indispensable role for the chemokine receptor CCR10 in IgA antibody-secreting cell accumulation. *J Immunol* **181**, 6309-6315.

16. Iwata M, Hirakiyama A, Eshima Y, *et al.* (2004) Retinoic acid imprints gut-homing specificity on T cells. *Immunity* **21**, 527-38.

17. Mora JR, Iwata M, Eksteen B, *et al.* (2006) Generation of gut-homing IgA-secreting B

cells by intestinal dendritic cells. *Science* **314**, 1157-1160.

18. Hieshima K, Kawasaki Y, Hanamoto H, *et al.* (2004) CC chemokine ligands 25 and 28 play essential roles in intestinal extravasation of IgA antibody-secreting cells. *J Immunol* **173**, 3668-3675.

19. Wurbel M, Phillipe J, Nguyen C, *et al.* (2000) The chemokine TECK is expressed by thymic and intestinal epithelial cells and attracts double- and single-positive thymocytes expressing the TECK receptor CCR9. *Eur. J. Immunol* **30,** 262-271.

20. Papadakis KA, Prehn J, Nelson V, *et al.* (2000) The role of thymus-expressed chemokine and its receptor CCR9 on lymphocytes in the regional specialization of the mucosal immune system. *J Immunol* **165**, 5069-5076.

21. Riaz-Ul-Haq, Pfahl M, Chytil F. (1991) Retinoic acid affects the expression of nuclear retinoic acid receptors in tissues of retinol-deficient rats. *Proc Natl Acad Sci USA* **88**, 8272-8276.

22. Takase S, Suruga S, Goda T. (2000) Regulation of vitamin A metabolism-related gene expression. *Brit J Nutr* **84**, **Suppl. 2**, S217-S221.

23. Szondy S, Reichert U, Fesüs L. (1998) Retinoic acids regulate apoptosis of T lymphocytes through an interplay between RAR and RXR receptors. *Cell Death Differ* **5**, 4-10. .

24. Lømo J, Smeland EB, Ulven S, *et al.* (1998) RAR-, not RXR, ligands inhibit cell activation and prevent apoptosis in B-lymphocytes. *J Cell Physiol* **175**, 68-77.

25. Statistical Analysis Systems (SAS) (1997) *SAS/STAT software: Changes and Enhancement Through Release 6.12* SAS Institute, Cary, NC.

26. Harris NL, Spoerri I, Schopfer JF, *et al.* (2006) Mechanisms of neonatal mucosal antibody protection. *J Immunol* **177**, 6256-6262.

27. Takagi N, Hasegawa S, Sugimoto M, *et al.* (2010) Effects of feeding whey protein or

skim milk protein on nitrogen utilization and fecal IgA in mice after weaning. *Kansai J Anim Sci* **166**, 1-9 (In Japanese, with English abstract).

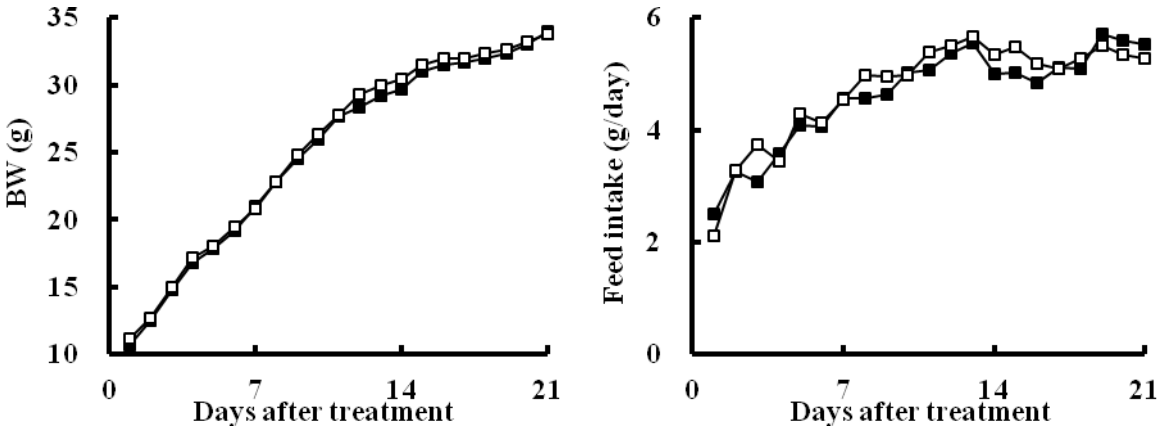
28. Garcia AL, Rühl R, Herz U, *et al.* (2003) Retinoid- and carotenoid-enriched diets influence the ontogenesis of the immune system in mice. *Immunol* **110**, 180-187.
29. Lorenz RG, Chaplin DD, McDonald KG, *et al.* (2003) Isolated lymphoid follicle formation is inducible and dependent upon lymphotoxin-sufficient B lymphocytes, lymphotoxin beta receptor, and TNF receptor I function. *J Immunol* **170**, 5475-5482.
30. Nakamura Y, Nosaka S, Suzuki M, *et al.* (2004) Dietary fructooligosaccharides up-regulate immunoglobulin A response and polymeric immunoglobulin receptor expression in intestinal of infant mice. *Clin Exp Immunol* **137**, 52-58.
31. Wang M, Sugimoto M, Ikeda S, *et al.* (2013) Effects of coumestrol administration to maternal mice during pregnancy and lactation on IgA-secreting cells in mammary gland. *Anim Sci J* 84, 322-327.
32. Allen LH & Haskell M (2002) Estimating the potential for vitamin A toxicity in women and young children. *J Nutr* 132, 2907S-2919S.

Table 1. The mRNA expression of IgA C-region, CCL25, retinoid X receptor (RXR) α, retinoic acid receptor **(**RAR) α and RARγ in jejunum and ileum of the control group after 7 (*n* 8), 14 (*n* 8) and 21 (*n* 6) days of treatment and the β-carotene group after 7 (*n* 8), 14 (*n* 8) and 21 (*n* 6) days of treatment.
(Mean values with their standard errors)

		Control		β-carotene		
	Days	Mean	SE	Mean	SE	*P* value
Jejunum						
IgA mRNA/GAPDH	7	0.79	0.13	0.87	0.12	0.660
	14	0.64	0.09	1.28	0.15	0.004
	21	1.06	0.07	1.49	0.08	0.007
CCL25 mRNA/GAPDH	7	1.06	0.06	1.23	0.21	0.469
	14	0.80	0.06	1.29	0.13	0.008
	21	1.00	0.04	1.35	0.12	0.029
RXRα mRNA/GAPDH	7	1.00	0.14	1.18	0.10	0.343
	14	0.98	0.11	1.07	0.13	0.671
	21	0.97	0.05	1.57	0.10	0.007
RARα mRNA/GAPDH	7	1.27	0.11	0.79	0.08	0.006
	14	1.00	0.07	1.13	0.11	0.379
	21	1.10	0.07	1.55	0.14	0.022
RARγ mRNA/GAPDH	7	1.20	0.13	0.90	0.08	0.093
	14	0.99	0.12	1.70	0.23	0.022
	21	1.00	0.07	1.68	0.20	0.014
Ileum						
IgA mRNA/GAPDH	7	0.71	0.07	0.62	0.06	0.430
	14	1.02	0.17	1.05	0.09	0.893
	21	1.34	0.42	1.37	0.21	0.965
CCL25 mRNA/GAPDH	7	1.00	0.06	0.99	0.16	0.941
	14	1.26	0.18	1.48	0.22	0.482
	21	1.18	0.32	1.27	0.21	0.836
RXRα mRNA/GAPDH	7	1.04	0.05	1.12	0.09	0.497
	14	1.12	0.09	1.29	0.13	0.314
	21	0.79	0.10	1.32	0.10	0.005
RARα mRNA/GAPDH	7	1.04	0.07	0.98	0.05	0.580
	14	1.41	0.11	1.09	0.11	0.065
	21	1.00	0.04	1.16	0.05	0.055
RARγ mRNA/GAPDH	7	1.13	0.08	1.27	0.14	0.443
	14	1.37	0.11	1.15	0.09	0.170
	21	1.30	0.07	1.61	0.08	0.026

GAPDH, glyceraldehyde-3-phosphate dehydrogenase
The mRNA expression is represented relative to the IgA C-region, CCL25, RXRα, RARα and RARγ mRNA expression normalised by abundance of GAPDH mRNA.

399



400

401 **Fig. 1.** Body weights (BW) and feed intake of the control (■; *n* 6) and β-carotene (□; *n* 6)

402 groups during 21days of treatment.

403

404

405

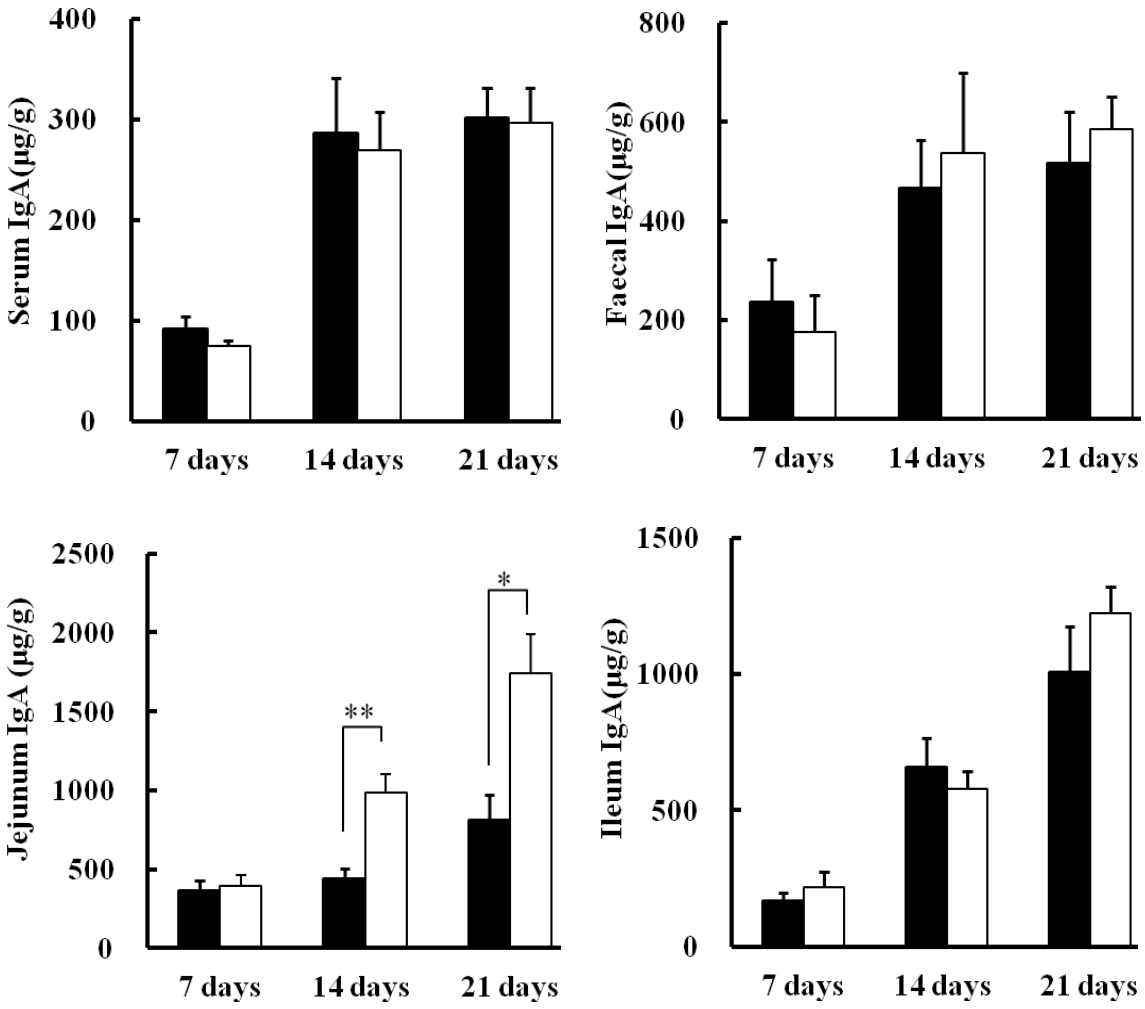


Fig. 2. IgA concentration (μg/g) in serum, jejunum, ileum and faeces of the control group (■) after 7 (*n* 8), 14(*n* 8) and 21 (*n* 6) days of treatment and the β-carotene group (□) after 7 (*n* 8), 14 (*n* 8) and 21 (*n* 6) days of treatment (Mean values with their standard errors). **Means between control and β-carotene groups were significantly different ($P<0.01$), *Means between control and β-carotene groups were significantly different ($P<0.05$).

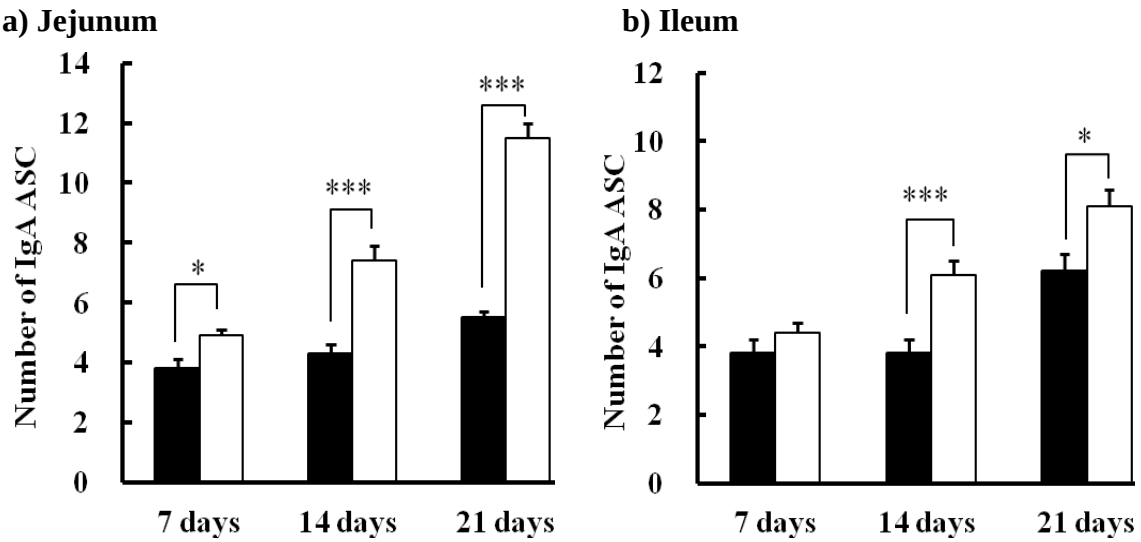


Fig. 3. The numbers of IgA antibody-secreting cells (ASC) in jejunum and ileum of the control group (■) after 7 (*n* 8), 14(*n* 8) and 21 (*n* 6) days of treatment and the β-carotene group (□) after 7 (*n* 8), 14(*n* 8) and 21 (*n* 6) days of treatment (Mean values with their standard errors).

The numbers of IgA ASC in jejunum and ileum were counted in lamina propria of villi in eight randomized villi from each mouse.

***Means between control and β-carotene groups were significantly different ($P<0.001$),

*Means between control and β-carotene groups were significantly different ($P<0.05$).